

REMARKS

Claims 49 and 58–101 are pending in this Application in view of Applicant’s Responses filed November 8, 2007, October 11, 2007 and September 17, 2007. Claims 49, 58-83 and 87-89 are hereby amended. No claims are added or cancelled.

Support for the amendments can be found throughout the specification and as discussed below.

No new matter has been entered.

The rejection under 35 U.S.C. § 112, 2nd paragraph (indefiniteness) is overcome

Claims 51-57 are rejected under 35 U.S.C. § 112, 2nd paragraph, as allegedly being indefinite for failing to point out and distinctly claim the subject matter which the Applicant regards as the invention.

The Office action specifically contends that the recitation “unfractionated samples of lysed blood” is unclear in light of the prosecution history in this application and in the parent applications from which this application claims priority.

Applicant notes that claims 51-57 were cancelled by way of Applicant’s Response filed on September 17, 2007, thereby rendering this rejection moot.

The rejections under 35 U.S.C. § 112, 1st paragraph (written description) are overcome

Claims 55-57 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. Applicant notes that claims 55-57 were cancelled by way of Applicant’s Response filed on September 17, 2007, thereby rendering this rejection moot.

Claims 63, 64, 65, 72, 73, 74, 81, 82, 83, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 and 101 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. The Office action states that the limitation “comprises leukocytes which have not been fractionated into cell types” is new matter. The Office action contends that the recitation includes the testing of whole blood, as well as, blood in which the red and white cells have been separated. The Office action states that only the first meaning, i.e., the testing of whole blood, has support in the application.

While Applicant disagrees with the rejection, the claims have been amended solely in the interest of advancing prosecution of the instant claims. Accordingly, each of

the rejected claims have been clarified to require the testing of a whole blood sample, wherein the leukocytes thereof have not been fractionated into cell types. This amendment clarifies that the blood that is tested must be whole blood. The Office action admits that there is indeed support for the testing of whole blood. See page 2, lines 14-15 of the Office action.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, first paragraph.

The rejections under 35 U.S.C. § 112, 1st paragraph (enablement) are overcome

Claims 49 and 58-101 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement for failing to provide in the specification a description that enables one skilled in the art to make and/or use the invention. The Applicant respectfully disagrees with the rejection and traverses as follows.

According to the Federal Circuit in *In re Wands*,

Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. 'The key word is undue, not experimentation.' The determination of what constitutes undue experimentation in a given case requires the application of standard of reasonableness, having due regard for the nature of the invention and the state of the art. The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed ... [Citations omitted].

Id. at 1404.

Against this background, determining whether undue experimentation is required to practice a claimed invention turns on weighing many factors summarized in *In re Wands*. For example, (1) the quantity of experimentation necessary; (2) the amount of direction or guidance presented; (3) the presence or absence of working examples of the invention; (4) the nature of the invention; (5) the state of the prior art; (6) the relative skill of those in the art; (7) the predictability or unpredictability of the art; and (8) the breadth of the claims. When all of the factors are given equal consideration in view of the instant

specification and pending claims, one must conclude that undue experimentation would not have been required to make and/or use the present invention.

Turning first to the nature of the invention and the scope of the claims, the present invention, as currently amended, is directed to various embodiments.

Independent claims 49 and 58-65 (and their related dependent claims) are directed to a method for *identifying* a human test subject *as being a candidate for having coronary artery disease (CAD)* comprising *quantifying* CRTAM RNA from a test subject's blood and then *comparing* the level of CRTAM RNA with the corresponding level of RNA in one or more test subjects that are either CAD+ or CAD-. A *statistically significant* difference in the levels of CRTAM RNA between the test subject and the controls, *wherein the difference is statistically significant at a threshold of p<0.05*, identifies that human test subject as being a candidate for having coronary artery disease.

Independent claims 66-74 (and their related dependent claims) are directed to a method for *classifying* a human test subject *as being more likely than not to have coronary artery disease (e.g., being more likely to have coronary artery disease than to be healthy)*, comprising *quantifying* CRTAM RNA from a test subject's blood and then *comparing* the level of CRTAM RNA with the corresponding level of RNA in one or more test subjects that are either CAD+ or CAD-. A *statistically significant* difference in the levels of CRTAM RNA between the test subject and the controls, *wherein the difference is statistically significant at a threshold of p<0.05*, classifies that human test subject as being more likely than not to have coronary artery disease.

Independent claims 75-83 (and their related dependent claims) are directed to a method for *detecting expression of a CRTAM gene* in a human test subject comprising *quantifying* CRTAM RNA from a test subject's blood and then *comparing* the level of CRTAM RNA with the corresponding level of RNA in one or more test subjects that are either CAD+ or CAD-. A *statistically significant* difference in the levels of CRTAM RNA between the test subject and the controls, *wherein the difference is statistically significant at a threshold of p<0.05*, classifies that human test subject as suspected of having coronary artery disease.

In view of the amendments to the claims, Applicant respectfully submits that neither the nature of the invention nor the scope of the claims gives rise to any

justification to conclude that the present invention would have required undue experimentation to make and/or use.

As presently amended and noted above, the instant invention is drawn to *identifying a human test subject as being a candidate for having coronary artery disease (CAD), classifying a human test subject as being more likely than not to have coronary artery disease (e.g., classifying a human test subject as being more likely to have coronary artery disease than to be healthy), or detecting expression of a CRTAM gene in a human test subject, based on comparing quantified levels of RNA measured from blood samples of test subjects with levels of RNA measured from at least control subjects having coronary artery disease, wherein a meaningful comparison requires a statistical significance to be met at the threshold of p<0.05.* While Applicant agrees with the Examiner that the claims generally relate to “*some diagnostic purpose*,” (see page 6, line 21, OA) if not distantly, none of the claims specifically recite, require or even seek to encompass “*a method for detecting coronary artery disease in a human test subject*,” as stated in the Office action (see page 5, line 15, OA). Instead, the claims, as now amended, are drawn specifically to *identifying a human test subject as being a candidate for having coronary artery disease (CAD), classifying a human test subject as being more likely than not to have coronary artery disease, or detecting expression of a CRTAM gene in a human test subject.* These methods are clearly not of the same scope as a method for detecting coronary artery disease.

This does not, however, diminish the utility of the methods claimed. All of the claims merely require that the determinations resulting from the comparisons within the claim result in some *indication* or even a *likelihood* of coronary artery disease in the tested subject relative to a likelihood of not having CAD (e.g., relative to a likelihood of being healthy). The claims do not claim, seek, or even require the absolute detection or diagnosis of coronary artery disease. The use of a biomarker, as is used in the present claims, as a type of indicator of a disease is typically just one aspect, and typically an early aspect, of a multi-factorial process used in diagnosing a person as having a particular disease of interest and can be useful in providing guidance in medical decisions regarding additional testing and treatment of a disease. The claimed methods are clearly not aimed at definitively providing a definitive diagnosis of coronary artery disease, but

rather, a useful approach aimed to provide assistance in the early stages of evaluating a patient for coronary artery disease, e.g., from a simple blood draw.

As noted in Stedman's 27th Edition Medical Dictionary, *indication* is not equated with *diagnosis*. The term *indication* is understood to mean "***the basis for initiation of a treatment for a disease or of a diagnostic test***" (see page 892). Even a *diagnostic test* is not considered to result in an absolute certainty of a diagnosis of disease – but rather is noted as "***relating to or aiding in diagnosis***". As noted in Harrison's Principles of Internal Medicine, Introduction to Clinical Medicine "the purpose of performing a test on a patient is to reduce uncertainty about the patient's diagnosis or prognosis and to aid the clinician in making management decisions" (Ch I, pg. 11). This same text further notes that while "a perfect test would have a sensitivity of 100% and a specificity of 100% and would completely separate patients with disease from those without it...there are no perfect tests, after every test is completed the true disease state of the patient remains uncertain" (Ch I, pg. 11). Accordingly, in view of the above, Applicant respectfully requests that the Examiner reconsider the nature and scope of the subject matter that is actually presently claimed. It is respectfully asserted, given that the nature and scope of the invention is directed to *identifying* a human test subject *as being a candidate for having coronary artery disease (CAD)*, *classifying* a human test subject *as being more likely than not to have coronary artery disease* (e.g., *classifying a human test subject as being more likely to have coronary artery disease than to be healthy*), or *detecting expression of a CRTAM gene* in a human test subject, one or ordinary skill in the art would not have required undue experimentation to make and/or use the present invention.

In evaluating the guidance and/or quantity of experimentation provided in the specification, the Office action states that "the invention requires knowledge of a reliable association between comparing CRTAM expression and the indication that coronary artery disease is present in a human." See page 6, lines 12-14, OA. More in particular, the Office action contends that "the specification does not provide adequate guidance to appraise one of ordinary skill in the art as to what levels of CRTAM gene expression must be observed to successfully conclude that coronary artery disease is present. Further, although the specification teaches there are differences in CRTAM levels in a coronary artery disease population versus a control patient population, the specification is

silent as to the nature of the “difference” in magnitude or direction. Thus, given the lack of teaching in the specification and the highly unpredictable nature of the technology, an extensive amount of work would be required to practice the claimed invention.” See page 12, lines 5-12. Contrary to the views taken in the Office action, Applicant respectfully submits that a reliable association is, in fact, to the extent needed, provided by both the specification, as well as, the post-filing evidence submitted in the Declaration of Dr. Jun Ma (“Declaration”) submitted in connection with the September 17, 2007 Response, in view of the presently amended claims. It is further submitted that, neither the magnitude or direction of the expression of the CRTAM, i.e., the “nature of the ‘difference,’” are absolutely required to enable, i.e., teach, one of ordinary skill in the art how to make and/or use the claimed invention. Instead, what is required is at least one method for enabling the invention, which is provided in the way of identifying statistically significant differentially expressed genes at a threshold of $p<0.05$.

Initially, Applicant wishes to emphasize that a specification is presumed to be an accurate disclosure of the claimed invention. In re Marzocchi, 439 F.2d 220, 224 (CCPA 1971). Further, post-filing date evidence can be used to verify the accuracy of a statement already in the specification. In re Brana, 51 F.3d 1560, 1567 n.19 (Fed. Cir. 1995). Additionally, the Office will appreciate that a considerable amount of experimentation is permissible to practice the claimed invention, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. See In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)). As asserted above, Applicant respectfully submits that by disclosing which genes are differentially expressed in blood of samples from individuals with coronary artery disease versus control subjects having coronary artery disease, Applicant has provided those of skill in the art one method, albeit not necessarily an exclusive method, to aid identifying a likelihood of coronary artery disease (e.g., relative to a likelihood of being healthy). It will be appreciated that as long as the specification discloses ***at least one method*** for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. § 112 is satisfied. See In re Fisher, 427 F.2d 833, 839 (CCPA 1970). Failure to disclose other methods (e.g., determination of magnitude

and/or direction) by which the claimed invention may be made does *not* render a claim invalid under Section 112. See Spectra-Physics, Inc. v. Coherent, Inc. 827 F.2d 1524, 1533 (Fed. Cir.), *cert. Denied*, 484 U.S. 954 (1987). Moreover, the fact that Applicant has exemplified specific genes which are differentially expressed with a p value of less than 0.05 from the tens of thousands of genes screened and with values that are often much lower than 0.05, is the nexus of the invention and knowledge of the direction or magnitude of the differential expression is not required to practice the claims.

A review of the specification and Declaration do not support a conclusion that undue experimentation would have been required to practice the invention. For instance, Example 31 of the specification describes an experiment done to demonstrate the use of the claimed invention to detect differential expression of genes in blood samples taken from subjects with coronary artery disease as compared to the blood of subjects who do not have coronary artery disease and who are otherwise healthy. The total mRNA from whole blood was taken from each test subject and control subject, converted to fluorescently labelled probes, and hybridized against an Affymetrix U133A Chip 39,000 human gene transcripts. Expression profiles were generated and analyzed using known software (GeneSpringTM). Of the 39,000 genes represented on the chip, 967 genes were identified as being differentially expressed with a p value of <0.05 between the coronary artery disease subjects and those subjects without coronary artery disease, each of which are shown in Table 3L. See Example 21.

This data is on its face certainly sufficient to establish a reliable association between differentially expressed CRTAM and the likelihood of coronary artery disease in a tested subject (e.g., relative to a likelihood of being healthy). The claims are directed to the detection of a differentially expressed CRTAM gene between a test subject and control CAD+ subject for *identifying* a human test subject *as being a candidate for having coronary artery disease (CAD)*, *classifying* a human test subject *as being more likely than not to have coronary artery disease* (e.g., *as being more likely to have CAD than to be healthy*), or *detecting expression of a CRTAM gene* in a human test subject. Indeed, the claims are not aimed at making a definitive diagnosis of coronary artery disease. Rather, their scope is aimed at an earlier stage in the disease diagnosis timeline. It is unnecessary for the data in the specification to expressly set forth magnitude and

direction of differential gene expression between the CAD and control subjects tested since this can be expected to be an inherent biological property of such subjects.

Indeed, methods and protocols for applying differentially expressed genes to indicate the presence of a disease or condition, *regardless of direction of change of expression*, are well established in the art and disclosed and incorporated by reference in the specification. For example, Slonim DK, Nature Genetics Supplement, Vol. 32, 502-8 (2002), which is incorporated by reference in the instant specification at paragraph 133 of the published application (2004/0265869), states that "*[t]he most basic question one can ask in a transcriptional profiling experiment is which genes' expression levels changed significantly.*" Applicant respectfully submits that Table 3L, in fact, provides which genes' expression levels—including CRTAM (p value of 0.029)—changed significantly; and thus, the specification comports with the methods and protocols generally accepted in the art. Submitted herewith is a copy of Slonim, which the Office is kindly asked to make of record.

Moreover, the Declaration further demonstrates that there exists a reliable association between CRTAM expression levels in subjects having CAD and healthy controls. The Declaration discloses post-filing date experiments performed by Dr. Jun Ma which continue to demonstrate that there is statistically significant ($p<0.05$) differential expression of CRTAM between patients having coronary artery disease and healthy control patients. And, the results of these experiments demonstrate that the average level of CRTAM-encoded RNA in blood samples from 19 coronary artery disease patients, as determined via quantitative RT-PCR, is about 2.9-fold higher than that of 14 healthy control subjects. The magnitude and directionality of the difference in levels of CRTAM RNA in blood of subjects having coronary artery disease relative to subjects not having coronary artery disease is an inherent biological feature of such subjects. Magnitude and directionality are thus inherent aspects of a statistically significant differentially expressed gene, and such features do not need to be expressly set forth in the specification or the claims to enable the claims.

Further, post-filing date evidence of the Declaration merely validate the teachings of the specification. As pointed out above, post-filing date evidence can be used to verify the accuracy of a statement already in the specification. In re Brana, 51 F.3d 1560, 1567

n.19 (Fed. Cir. 1995). The Applicant respectfully submits that the invention is taught by the specification and claimed in such terms that one skilled in the art can make and use the claimed invention, including the use of the elected biomarker, CRTAM, as an indicator of a likelihood of coronary artery disease in a tested subject (e.g., relative to a likelihood of being healthy) without the a priori need to know the direction or the level of differential expression that exists between subjects having coronary artery disease and subjects not having coronary artery disease.

Lack of directionality and/or magnitude in the specification as-filed does not render the claims of the present scope non-enabled. The Applicant has identified the elected gene CRTAM as differentially expressed between individuals diagnosed as having coronary artery disease and healthy controls by demonstrating a statistically significant difference in the level of RNA, as described in Example 21. The statistical significance of CRTAM's differential expression is evidenced by its p value of 0.029 as listed in Table 3L, acknowledged by the Office Action. The dendrogram of Figure 19 demonstrates that the CRTAM gene is one of a number of genes which demonstrate a statistically significant difference between individuals who have coronary artery disease and healthy individuals. Therefore the Applicant has taught that there is a significant difference in differential expression for CRTAM between a population of individuals having coronary artery disease and a population of healthy individuals, and further has taught to compare the level of expression of CRTAM in a test individual with populations having coronary artery disease and populations of healthy subjects using methods to determine the similarity or difference in gene expression levels between the test subject and the tested control populations.

Support for such teachings can be found in the published application, for example at paragraphs [0126] to [0127] (relating to "...*comparing two or more samples for differences...*"), paragraph [0128] (relating to "*Identification of genes differentially expressed in blood samples from patients with disease as compared to healthy patients or as compared to patients without said disease...*"), [0133] (relating to utilization of "...*sets of genes which have been identified as statistically significant*" in order to "...*characterize an unknown sample as having said disease or not having said disease...*") for class prediction, [0134] (relating to "*Methods that can be used for class*

prediction analysis...”), [0135] (relating to use of algorithms which “...can be used to query additional samples against the existing database to further refine the diagnostic and/or prognostic determination by allowing an even greater association between the disease and gene expression signature...”), and [0367] (relating to “Identification of genes differentially expressed in blood samples from patients with Coronary artery disease as compared to healthy patients... by statistical analysis using the Wilcoxon Mann Whitney rank sum test ...”).

And, as particularly emphasized above, magnitude and/or directionality are not necessarily the key and/or exclusive determination that is needed to be made. Instead, **“[t]he most basic question one can ask in a transcriptional profiling experiment is which genes’ expression levels changed significantly.”** (Slonim DK, see above).

Magnitude and/or directionality are inherent features of the expression level of a gene which has been determined with statistical significance to be differentially expressed in diseased subjects versus healthy subjects. Accordingly, the Applicants believe that the specification and Declaration, alone or together, establish there exists the requisite reliable association between CRTAM expression levels in subjects having coronary artery disease and healthy controls.

Turning to the unpredictability factor, Applicant respectfully submits that the purported unpredictability in the art does not give rise to any justification to conclude that the present invention, as instantly claimed, would have required undue experimentation to make and/or use. In addressing the unpredictability factor, the Office action points to a number of references which apparently generally refer to the unpredictability in the field of large-scale gene expression analysis and disease prediction. In particular, the Office action states that Lee teaches that data obtained from gene chips must be replicated in order to screen out false positive results; Cheung et al. (2003) teaches that there is natural variation in gene expression amongst different individuals; Wu et al (2001) teaches that gene expression data, such as microarray data, must be interpreted in the context of other biological knowledge, and that the conclusions that can be drawn from a given set of data depend on the particular choice of data analysis; and Newton et al. (2001) teaches that a replication of data is required for validation (see pages 9-12, OA).

First, Applicant respectfully asks that the Examiner reconsider the scope of the present invention as instantly claimed. The claims are not directed to a method for definitively diagnosing coronary artery disease based on detecting differentially expressed biomarkers. Instead, the scope of the claims is aimed at steps occurring earlier in the disease diagnosis timeline. In particular, the instant invention is drawn to *identifying a human test subject as being a candidate for having coronary artery disease (CAD), classifying a human test subject as being more likely than not to have coronary artery disease (e.g., as being more likely to have coronary artery disease than to be healthy), or detecting expression of a CRTAM gene in a human test subject, based on comparing quantified levels of RNA measured from blood samples of test subjects with levels of RNA measured from at least control subjects having coronary artery disease, wherein a meaningful comparison requires a statistical significance to be met at the threshold of p<0.05.* Clearly, none of these methods claim the diagnosis or absolute detection of a coronary artery disease.

In view of the Examiner's specific concerns regarding Lee; Cheung et al.; Wu et al; and Newton et al, as described above, it is respectfully submitted that the Declaration clearly demonstrates, using additional samples and additional techniques (e.g., quantitative RT-PCR), that CRTAM is a statistically significant differentially expressed gene between individuals having coronary artery disease and healthy individuals.

Furthermore, Applicant would like to point out that the results disclosed by Cheung *et al.* cannot be reliably extrapolated to primary blood samples since the lymphoblastoid cells employed by Cheung *et al.* are significantly modified relative to primary blood cells, due to being cultured cell lines generated by immortalization of primary human cells derived from "CEPH" families, as indicated in Reference no. 10 of Cheung *et al.* (Dausset *et al.*, 1990. Genomics 6:575; enclosed) at p. 575, right column, 1st paragraph. Applicant wishes to point out that immortalized cultured cell lines such as the lymphoblastoid cells taught by Cheung *et al.* undergo significant genetic modification such as strong genome-wide demethylation (refer, for example, to enclosed abstract of: Vilain *et al.*, 2003. DNA methylation and chromosome instability in lymphoblastoid cell lines. Cytogenet Cell Genet. 90:93), as a result of extensive *in-vitro* culturing in the absence of immune or apoptotic mechanisms which function to eliminate

mutated cells in the body. As such, immortalized CEPH lymphoblastoid cells may represent a particularly unsuitable cell type for modeling gene expression variability in primary blood cells.

Applicant would also respectfully disagree with the contention in Wu *et al.* that expression data needs to be interpreted in view of other biological knowledge.

Differential gene expression which is reproducible and is correlated with the state of health or disease of the individual does not necessarily result directly from the state of disease of the individual. Rather these changes in expression can be as a result of a downstream effect of pathogenic processes, and it is not necessary that the biological relevance of the data be known to allow this difference in expression to be useful as a biomarker. In other words, no specific understanding of the role of CRTAM or its involvement in coronary artery disease is required in the specification to enable the claims. For example prostate-specific phosphatase and prostate-specific antigen (PSA) were long used as biomarkers without an understanding of their function. For example, reference can be made to the enclosed abstracts of: Chu TM, 1990, Prostate cancer-associated markers. Immunol. Ser. 53:339-56; and Diamandis EP., 2000. Prostate-specific antigen: a cancer fighter and a valuable messenger? Clin Chem. 46:896-900.

Applicant also takes issue with Loughran JR et. al., which notes a differential expression of either 3.9 fold or 11.8 fold for CRTAM of LGL patients compared with healthy individuals. Loughran, however, **only looks at differential expression of fractionated blood samples**, either in mononuclear cells (PBMCs) (see for example p.8 para. [0063], [0066]; p. 9 para. [0075] and [0076] and Table 2) or further fractionated leukocytes, namely, CD8+ T cells (see.p.8 para.[0066] and p.9 para [0077]). This is in contrast to the experiments performed by the Applicant which utilize RNA from whole blood samples which include RNA of all leukocytes, without any fractionating (paragraph [0240] of the Published Application). As noted in post-filing reference Du et al., each of the blood cell types can provides its own unique contribution towards a measured level of expression between disease and control subjects (“several blood RNA isolation methods have been used to date...however, the RNA isolated using these methods comes from various blood cell subsets that originate from different developmental lineages, perform separate and distinct biological functions, and, most

likely, have very different genomic expression signatures" (see p.701, 1st column of Du et al.), therefore it is not known what the level of difference in expression would be in RNA from samples as claimed when comparing patients with LGL to control individuals and whether there would be a statistically significant similar level of expression as between patients having LGL and patients having coronary artery disease.

The Office action also contends that the "*there is no analysis of all possible diseases or phenotypes to determine if the gene expression difference observed in the instant application is specific to coronary artery disease such that any difference between a test patient and blood samples from control subjects is sufficient to conclude coronary artery disease is present*" (page 9 of the Office Action). The Office Action cites Loughran, JR et al. as observing that "*CRTAM is differentially expressed in patients having large granular lymphocyte leukemia as compared to healthy patients*" (US 2007/0020666, p.23), and Zlontnik et al. as teaching that CRTAM is expressed specifically on activated class I MHC-restricted T cells (p. 9-10 of the Office Action).

Applicant respectfully submits that the claims are not directed to the identification of an indication or a likelihood of any other disease other than coronary artery disease. The CRTAM marker might very well be present in diseases other than coronary artery disease. The fact that CRTAM might be involved in other diseases is irrelevant, however, because the claims concern only coronary artery disease. Thus, Applicant respectfully disagrees with the Examiner that the instantly claimed invention requires that coronary artery disease is present to the exclusion of all other possible diseases or phenotypes.

For instance, the Examiner suggests that differential expression of CRTAM RNA could be an indicator of an immune response based on Zlontnik's teachings that CRTAM protein is expressed on activated T-cells. First, CRTAM expression may very well be a component of an immune response and such is not excluded or required by the claims. The claims, instead, are directed to identifying statistically significant differentially expressed CRTAM as a biomarker to indicate that a subject may have or be a candidate having coronary artery disease. In addition, the claims require detection of the expression in whole blood. Zlontnik's work is reported with respect to only activated T-cells. As noted above, an increase in activated T-cells is only one of the cell types

expressing RNA in whole blood. The entire teachings of Zlontnik are limited to demonstrating an increased cell surface expression of a protein on the surface of activated T-cells. The level of surface expression of a protein on a T-cell is not necessarily even a good or accurate measure of the expression level of a protein since increased surface expression of the protein may merely be due to increased transport of the protein to the cell surface. Examples of such regulation abound in the art.

The Office action also suggests that the Applicant has not taught that the elected gene alone is sufficient to indicate the presence of coronary artery disease. Applicant notes that by virtue of demonstrating a differential expression between individuals having coronary artery disease and healthy individuals, they have demonstrated that the single gene is indicative of a likelihood of coronary artery disease (e.g., relative to a likelihood of being healthy), as now claimed. Furthermore, the Applicant has demonstrated, both within the specification, and in the Declaration that the differential expression of the elected gene is statistically significant between individuals having coronary artery disease and healthy individuals itself demonstrating that the elected gene is indicative of a likelihood of coronary artery disease (e.g., relative to a likelihood of being healthy). Finally, Applicant notes that it is not aware of any teaching or suggestion of analyzing blood for biomarkers of coronary artery disease prior to Applicant's filing.

In short, the Examiner appears to be asking the Applicant to enable a "gold standard" diagnostic test (i.e., the most definitive method available to establish the presence of a disease) (see Bachmann L.M. et al. at p.953 enclosed). A gold standard diagnostic test is often expensive and/or not possible without invasive surgery, and in some cases is only available post mortem. This does not diminish the utility of the methods claimed. As already noted above, the use of a biomarker as an indicator that a disease may be present, is typically just one aspect of a multi-factorial process used for determining the likelihood that the person may have the disease of interest and so as to guide medical decisions regarding additional testing and treatment. An *indication* is not the same as a *diagnosis*. As previously mentioned, an *indication* is understood to mean "**the basis for initiation of a treatment for a disease or of a diagnostic test**" and is not meant to provide an absolute certainty of diagnosis. See *supra*.

The claims in their present form are not aimed at an absolute method of diagnosis. For example, claims 66-74 are directed to a method for *classifying* a human test subject *as being more likely than not to have coronary artery disease (e.g., as being more likely to have coronary artery disease than to be healthy)*, comprising *quantifying* CRTAM RNA from a test subject's blood and then *comparing* the level of CRTAM RNA with the corresponding level of RNA in one or more test subjects that are either CAD+ or CAD-. A *statistically significant* difference in the levels of CRTAM RNA between the test subject and the healthy controls, *wherein the difference is statistically significant at a threshold of p<0.05*, classifies that human test subject as being more likely than not to have coronary artery disease. This claim is far removed from a method of detecting a disease. The claim merely provides, in a binary fashion, a way to classify whether a test subject is more likely than not to have coronary artery disease. This is not a detection of coronary artery disease. Nevertheless, the claim is not without utility.

As stated in the Manual of Patent Examining Procedure at 2164.03: the “predictability or lack thereof” in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. In this case the disclosed result is a statistically significant difference in the level of CRTAM RNA as between individuals having coronary artery disease and healthy individuals. The claimed invention requires a statistically significant similarity between the level of expression of CRTAM between the test subject and individuals having coronary artery disease and a statistically significant difference as between the test subject and individuals not having coronary artery disease, such as healthy individuals. These results are confirmed by the Declaration. Contrary to the view taken in the Office action, the Declaration does not “add” to the specification, but merely validates the data in the specification. One skilled in the art can *readily anticipate* utilizing similar experiments to those described in the specification, and applied in the Declaration, to the claimed invention, without undue experimentation.

At least in view of the above facts, as applied against the factors summarized in In re Wands, i.e., (1) the quantity of experimentation necessary; (2) the amount of direction or guidance presented; (3) the presence or absence of working examples of the invention; (4) the nature of the invention; (5) the state of the prior art; (6) the relative skill of those

in the art; (7) the predictability or unpredictability of the art; and (8) the breadth of the claims, the instantly claimed invention would not have required undue experimentation for one of ordinary skill in the art to make and/or use the invention.

In light of the amendments and above remarks, the Applicant contends that the claims are enabled, and respectfully requests reconsideration and withdrawal of the instant rejections.

CONCLUSION

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. No new matter is added. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

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Respectfully submitted,

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Encl.

- (a) Excerpts from Stedman, Thomas Lathrop, 1853-1938, Stedman's Medical Dictionary 27th (submitted with September 17, 2008 Response)
- (b) Excerpts from Harrison's Principles of Internal Medicine, ch I Introduction to Clinical Medicine, p. 11 (submitted with September 17, 2008 Response)
- (c) Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds. Fig. A.23 (submitted with September 17, 2008 Response)
- (d) Backmann L.M., Juni, P., Reichenback, S., Ziswiler, H-R, Kessels, A.G., and Vogelin, E. "Consequences of different diagnostic 'gold standards' in test accuracy research: Carpal Tunnel Syndrome as an example." *International Journal of Epidemiology* 2005 **34** 953-955 (submitted with September 17, 2008 Response)
- (e) Slonim D.K., From patterns to pathways: gene expression data analysis comes of age, Nature Genetics Supplement, 2002, 32:502-508 (submitted herewith)

Abstract of: Casey et al., 1988. simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9

Routine fixation and paraffin embedding destroys many hematopoietic and lymphoid differentiation antigens detected by flow cytometry or frozen section immunohistochemistry. On the other hand, morphologic evaluation is difficult in flow cytometric or frozen section studies. A simplified three-step plastic embedding system using acetone-fixed tissues embedded in glycol-methacrylate (GMA) resin has been found to provide both excellent morphologic and antigenic preservation. With our system, a wide variety of antigens are detected in plastic sections without trypsinization or prolonged embedding procedures; **pan-B (CD19, CD22), pan-T (CD7, CD5, CD3, CD2), T-subset (CD4, CD8, CD1, CD25) markers** as well as surface immunoglobulin and markers for myeloid and mononuclear-phagocyte cells are preserved. In summary, modifications of plastic embedding techniques used in this study simplify the procedure, apparently achieve excellent antigenic preservation, and facilitate evaluation of morphologic details in relation to immunocytochemical markers.

Abstract of: Vilain A., Bernardino J., Gerbault-Seureau M., Vogt N., Niveleau A., Lefrancois D., Malfoy B., and Dutrillaux B. (2000) DNA methylation and chromosome instability in lymphoblastoid cell lines. Cytogen. Cell Genet. (2000) 90 (1-2) 93-101

In order to gain more insight into the relationships between DNA methylation and genome stability, chromosomal and molecular evolutions of four Epstein-Barr virus-transformed human lymphoblastoid cell lines were followed in culture for more than 2 yr. The four cell lines underwent early, strong overall demethylation of the genome. The classical satellite-rich, heterochromatic, juxtacentromeric regions of chromosomes 1, 9, and 16 and the distal part of the long arm of the Y chromosome displayed specific behavior with time in culture. In two cell lines, they underwent a strong demethylation, involving successively chromosomes Y, 9, 16, and 1, whereas in the two other cell lines, they remained heavily methylated. For classical satellite 2-rich heterochromatic regions of chromosomes 1 and 16, a direct relationship could be established between their demethylation, their undercondensation at metaphase, and their involvement in non-clonal rearrangements. Unstable sites distributed along the whole chromosomes were found only when the heterochromatic regions of chromosomes 1 and 16 were unstable. The classical satellite 3-rich heterochromatic region of chromosomes 9 and Y, despite their strong demethylation, remained condensed and stable. Genome demethylation and chromosome instability could not be related to variations in mRNA amounts of the DNA methyltransferases DNMT1, DNMT3A, and DNMT3B and DNA demethylase. These data suggest that the influence of DNA demethylation on chromosome stability is modulated by a sequence-specific chromatin structure. Copyright 2000 S. Karger AG, Basel.

Abstract of: Chu TM, Prostate Cancer-Associated Markers (1990) Immunol. Ser. 53:339-56.

Immunodiagnosis of prostate cancer is at a more advanced stage than that of most other tumors. Two well-known markers, prostatic acid phosphatase and prostate-specific antigen, have been used in the clinical management of patients. Prostate-specific antigen is a more sensitive and reliable marker than prostatic acid phosphatase. Serum prostate-specific antigen is effective in monitoring disease status, predicting recurrence, and detecting residual disease. Prostate-specific antigen is a tool for the histological differential diagnosis of metastatic carcinomas, especially in the identification of metastatic prostate tumor cells in distant organs and in the differentiation of primary prostate carcinoma from poorly differentiated transitional cell carcinoma of the bladder. Few data on biological function are available. Prostatic acid phosphatase functions as a phosphotyrosyl-protein phosphatase and prostate-specific antigen as a protease. Physiological function in the prostate remains to be elucidated. Several of the prostate-specific and prostate-tumor-associated antigens, as well as a putative prostate tumor-specific antigen, as recognized by monoclonal antibodies are available. Clinical evaluation of these potential markers is not yet available.

Abstract of Diamandis EP Prostate-specific antigen: a cancer fighter and a valuable messenger? Clin Chem. 2000 Jul; 46(7) 896:900

BACKGROUND: Prostate-specific antigen (PSA) is a valuable prostatic cancer biomarker that is now widely used for population screening, diagnosis, and monitoring of patients with prostate cancer. Despite the voluminous literature on this biomarker, relatively few reports have addressed the issue of its physiological function and its connection to the pathogenesis and progression of prostate and other cancers.

APPROACH: I here review literature dealing with PSA physiology and pathobiology and discuss reports that either suggest that PSA is a beneficial molecule with tumor suppressor activity or that PSA has deleterious effects in prostate, breast, and possibly other cancers. **CONTENT:** The present scientific literature on PSA physiology and pathobiology is confusing. A group of reports have suggested that PSA may act as a tumor suppressor, a negative regulator of cell growth, and an apoptotic molecule, whereas others suggest that PSA may, through its chymotrypsin-like activity, promote tumor progression and metastasis. **SUMMARY:** The physiological function of PSA is still not well understood. Because PSA is just one member of the human kallikrein gene family, it is possible that its biological functions are related to the activity of other related kallikreins. Only when the physiological functions of PSA and other kallikreins are elucidated will we be able to explain the currently apparently conflicting experimental data.